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FOLLICLE-STIMULATING HORMONE ACTIVATES MITOGEN-ACTIVATED PROTEIN KINASE IN PRE-NEOPLASTIC AND NEOPLASTIC OVARIAN SURFACE EPITHELIAL CELLS

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Introduction

Most ovarian tumors appear to arise from the ovarian surface epithelium (OSE), which is a simple squamous-to-cuboidal mesothelium covering the ovary. Recently, we established immortalized OSE (IOSE) cell lines, which are IOSE-29 (pre-neoplastic) and IOSE-29EC (neoplastic and tumorigenic), from normal OSE directly by transfection with simian virus 40 (SV40)-large T antigen and subsequent E-cadherin[1,2]. Considering that FSH plays a role in ovarian cell in terms of cell growth[3], we sought to investigate the effect of FSH in normal OSE and IOSE cell lines.

Materials and Methods

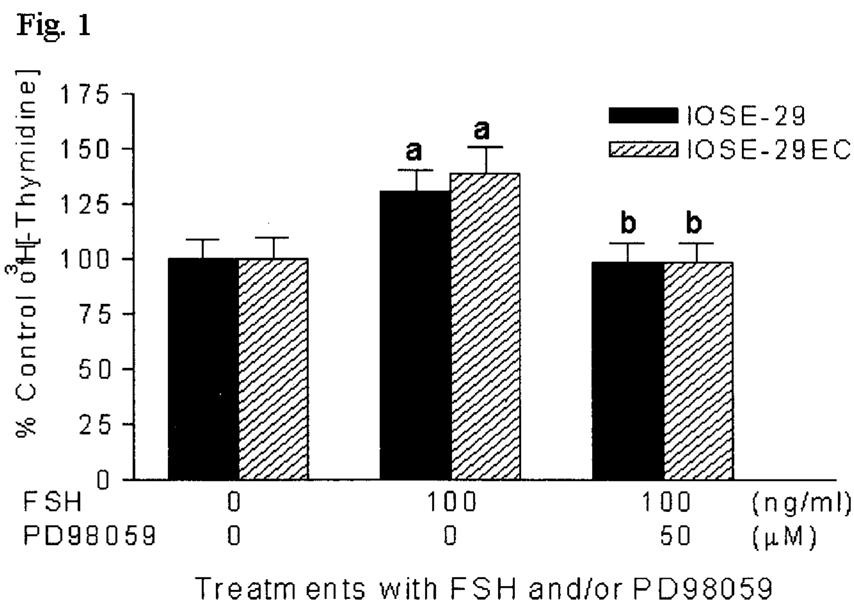
Normal human OSE cells were scraped from the ovarian surface during laparoscopies for nonmalignant disorders and cultured as previously described[4]. The non-tumorigenic SV40 Tag-immortalized OSE-derived line (IOSE-29), its tumorigenic derivatives (IOSE-29EC) and the cell lines derived from IOSE-29EC-inoculated SCID mice (IOSE-29EC/T4 and IOSE-29EC/T5) were cultured as described[2]. The ovarian carcinoma cell lines OVCAR-3 and SKOV-3 cells were cultured and used for the following experiments. To investigate the presence of FSH-R mRNA, a semi-quantitative PCR amplification after RT and Northern blot analysis were performed. In order to examine the effect of FSH on the cell growth, serum starved cells were treated with increasing concentrations (10, 100 and 1000 ng/ml) of FSH and a [^{3}H]thymidine incorporation assay was performed. In addition, the effect of FSH in the presence or absence of PD98059 was examined to elucidate whether MAPK activation by FSH may be related with growth-stimulation. The effect of FSH on MAPK activation was investigated by treating serum-starved cells with FSH in the presence or absence of PD98059 (50 mM) in a time and/or dose dependent manner. After treatments, level of the phosphorylated p44/p42 MAPK (P-MAPK, Thr^{202}/Tyr^{204}) was measured and normalized against the level of total p44/42 MAPK (T-MAPK, phosphorylation-state independent). *In vitro* MAPK assays were performed using

immunoprecipitated P-MAPK from cellular proteins and the Elk-1 fusion protein (as a substrate for activated MAPK), according to the manufacturer's suggested procedure (New England Biolab).

Results and Discussion

The presence of FSH-R was confirmed by RT-PCR and Northern blot analysis. Treatments with increasing doses of FSH (10, 100 or 1000 ng/ml) resulted in a significant growth-stimulation in normal OSE, OVCAR-3, IOSE-29 and IOSE-29EC cells. FSH induced a significant increase in MAPK activation in IOSE-29 and IOSE-29EC cells. The stimulatory effect of FSH was completely reversed by pretreatment with PD98059 in IOSE-29 and IOSE-29EC cells, confirming the activation of MAPK pathway cascade. Treatment with FSH appeared to induce a significant increase in MAPK activation in OVCAR-3 cells, whereas, no difference was observed in SKOV-3 cells following FSH treatment. Treatment with FSH resulted in a significant increase in Elk-1 phosphorylation, whereas pretreatment with PD98059 significantly inhibited FSH-induced Elk-1 phosphorylation in both IOSE-29 and IOSE-29EC cells. This results indicate that activated MAPK by FSH play a role in activating downstream transcription factors. FSH (100 ng/ml) resulted in a significant growth-stimulation in these cells as expected (Fig. 1). In addition, as seen in Fig. 1, pretreatments with PD98059 attenuated completely FSH-stimulated cell growth in both IOSE-29 and IOSE-29EC cells, indicating FSH-induced MAPK activation may mediate the growth effect of FSH in these cells. In conclusion, we demonstrated that FSH-R was expressed and FSH induced a growth-stimulation in normal, pre-neoplastic and neoplastic OSE cells. In addition, treatment with FSH resulted in an activation of MAPK cascade and activated MAPK phosphorylated Elk-1 in IOSE-29 and IOSE-29EC cells.

Fig. 1



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